mm. On the inside there is a corresponding very slight protuberance, becoming more conspicuous and transparent as the animal grows and, finally, showing a perforation. In this stage ovigerous females were found tightly wedged in the central cavity of the cyst. Later, the perforation becomes larger and the animal is liberated into the inside of the bladder. Since most bladders have areas eroded open to the exterior, the copepods, which tend to crawl by means of their swimming legs on the surface of the alga, probably infect neighboring plants.

No attempt has been made in this study to determine the mode of reproduction, whether parthenogenetic or, possibly, by external fertilization.

About 70–80 percent of the algae at the type locality were infected. Considering the degree of infection in individual thalli there seems to be an "all or none" effect, i.e., thalli are either free from copepods or are infected to a high degree. In one instance, 33 cysts per cm^2 were counted. It seems plausible that a population in a young frond, having very few perforations, is relatively confined and multiplies rapidly. This heavy infection in most cases leads to extensive perforations of the bladder.

The habitat in the alga, as described here, is a truly admirable one. The bladders hold water for a long time, even if exposed, or stay moist due to their relatively fleshy walls. Furthermore, there does not seem to be any predator of any consequence in or on the algae.

The name *cystoecus* (cyst dwelling) suggested itself as the most appropriate one.

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MALACOLOGY.—*A study of the shell structure and mantle epithelium of* Musculium transversum (*Say*).

Samuel W. Rosso, U. S. Navy Hydrographic Office.[1] (Communicated by Willis L. Tressler.)

Musculium transversum (Say) is a member of the family Sphaeriidae, a group of fresh-water bivalves that are worldwide in distribution and are found in lakes, pools, and rivers, where they occupy the shallowest of water and the lower depths of the deepest lakes. Some of the usually accepted characteristics of the Sphaeriidae include the presence of punctae in the shell material and the absence of a prismatic layer as a component of the shell. Previous studies have described the shell as consisting of only the nacreous layer and the periostracum, thereby differing from the other bivalves in which the shell consists of the periostracum, prismatic layer, and the nacreous layer.

This report is mainly concerned with the general shell structure of *M. transversum*, and evidence is presented that shows the presence of a prismatic layer as a natural component of the shell. A gross study of the mantle epithelium is also included, and a correlation of the shell structure with the mantle epithelium is attempted. The pyramidal cells, however, which occupy the punctae or shell canals, are treated lightly, and conclusive evidence as to their function is still lacking.

Schröder (1906–1907) cited Leydig as stating that the shell of *Sphaerium corneum* lacked the prismatic layer, the shell being composed only of the nacreous layer and periostracum. Leydig concluded that the punctae were hollow canals; that they were unbranched and measured 0.024 mm in length and were 0.003 mm wide; and that the mantle epithelium was composed of large cells, 0.007 mm to 0.012 mm, some of which grew into the canals. Leydig surmised that the purpose of the canals was similar to bone and tooth canals in that the purpose is to carry food materials.

[1] The author wishes to express his appreciation to Dr. Ellinor H. Behre, Louisiana State University, under whose direction this work was conducted. Special thanks are also due Dr. Harold Harry, Rev. H. B. Herrington, Dr. J. P. E. Morrison, Dr. H. E. Wheeler, Alan Cheetham, and Jesse West for their advice and assistance.

Schröder (loc. cit.) studied the structure of the shell and mantle epithelium of *Musculium lacustre*; he seemed to follow Leydig in that the shell lacked the prismatic layer. He described the mantle epithelium as consisting of (1) large, flat polygonal cells with conspicuous spherical nuclei, mostly peripheral in location, and (2) pyramidal cells with irregular bases located between the large polygonal ones. The tips of the pyramidal cells appeared as if drawn out into long processes that measured 0.003 mm to 0.060 mm in length, depending, of course, on the thickness of the shell; these processes extended through the shell canals and ended at the periostracum. He also recognized a finely coiled thread inside these processes which ended either on the periostracum or on a sort of a button above the periostracum. He thought that the cells might have a glandular function, but when he considered their position this assumption was rejected, mainly because it would seem strange to interpret them as secretory since they were not located at the edge of the mantle. Schröder did not deny the possibility that the cells were sensory in function; however, his work was interrupted before he observed any supporting evidence on this detail.

Schröder (loc. cit.) also pointed to the mantle papillae of the brachiopods, and cited Von Blochman, who surmised that the papillae were concerned with the secretion of the shell. Schröder did not speculate, however, on the extent to which the brachiopod papillae could be compared with the pyramidal cells of *Musculium*.

Oveinnikov (1931), in studying the shell structure of *Sphaerium corneum*, stated in agreement with the above workers that the punctae do not go through the shell to the exterior.

MATERIALS AND METHODS

Sections of the shell used in the study were prepared by a method long used by paleontologists in studying the structure of various fossils. A mounting medium, known as Lakeside 70, was melted on a glass microscope slide. A small hotplate was used for heating purposes. One of the valves or a shell fragment was set upright in the medium, and two pieces of matchsticks were used to stabilize the valve or fragments in upright positions. The slide was then removed from the hotplate, and the Lakeside 70 was allowed to cool and harden. Then the shell was ground, with a circular motion, against a flat piece of glass pane onto which moistened grinding powder (#600) has been placed. After a smooth, flattened surface was fashioned, the slide was washed off, dried, and placed back on the hotplate to remelt the Lakeside 70. Following this the matchsticks were removed and the ground, flattened surface of the shell was turned over and placed flat against the surface of the slide. The latter was removed from the hotplate, and the mounting medium was allowed to re-cool. The unground side of the valve or shell fragment was then ground with a circular motion, as before, against the glass surface. While the fragment or valve was being thinned down, frequent cleaning and examination of the object was necessary to prevent grinding the section too thin. When the grinding was completed and the slide thoroughly cleaned with running water, and dried, a cover-slip was cemented with Permount over the section to prevent any possible injury.

These ground sections were studied with compound microscope and polarizing microscope. The polarizing microscope was necessary for determining the orientation of the calcite crystals of the shell.

OBSERVATIONS AND DISCUSSION

Mantle epithelium and punctae.—Gross examination of the young clams and embryos of *M. transversum* showed that the pyramidal cells of the mantle send projections through the thin embryonic shell (see Fig. 1). Individual embryos and young were of varying sizes when the projections first appeared. The largest individual studied (an extramarsupial embryo)[2] in which the projections had not yet appeared measured 1.63 by 1.25 mm (length by height); the smallest individual measured with these projections was a 1.29 by 1.02 mm young clam.

Projections from the mantle epithelium appear first as slender rods, and those projections measured ranged in length from 0.009 to 0.045 mm, whereas the diameters varied from 0.003 to 0.009 mm.

Shortly after the projections appear on the surface of the shell, the thin, almost transparent shell becomes opaque, and the surface of the valves acquires a wrinkled appearance. This wrinkled appearance is due to formation of raised, polygonal areas, each having a projection in the center or near the edge. These areas are

[2] According to Okada (1935), an extramarsupial embryo is one that has been liberated from its marsupial sac and is capable of free movement about the inner branchial chamber.

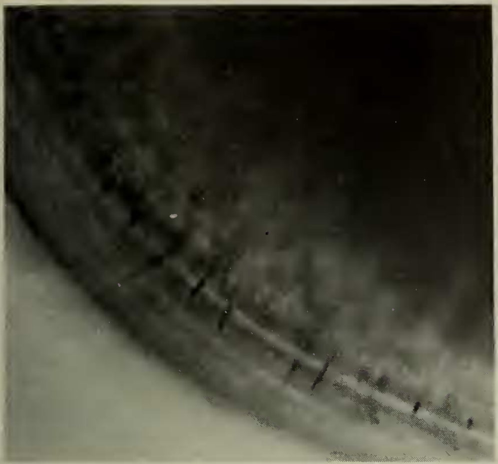

Fig. 1.—View of exterior surface of a young clam less than 3 mm in length. Note the projections along the ventral margins of the valve.

thought to be the bases of the crystal structure of the calcareous material of the shell. (This will be discussed later).

Examination of the ground sections of the adult and young shells shows that the shapes and sizes of the punctae closely resemble the projections that were described above. The openings of the punctae on the innermost surface (see Fig. 2) prove to be much wider than the slender, tubular portions of the punctae, with those measured varying from 0.012 to 0.018 mm. The slender, tubular portions of the punctae measured 0.003 to 0.009 mm in diameter (see above measurements of the diameters of the epithelial projections), thereby duplicating the diameters of the projections which they actually enclose. Lengths of the punctae vary with the thickness of the shell, with the larger adult shells being the thickest. These measurements also closely resemble the measurements of the pyramidal cells and punctae of *Sphaerium corneum* and of *M. lacustre* (see above).

Relationships of the punctae with the periostracum seem to vary with the individual punctae. Some terminate in raised plugs near the top of the periostracum, while others appear to end as pointed projections in the periostracum. Only a few extend up above the periostracum, but they seem to be covered with periostracum even though they extend above its general level. In a given plane of section, parts of punctae can be seen at different levels, which is due to the curvature of some of the punctae.

Shell structure.—Study of the ground sections of the shell of *M. transversum* shows that the shell consists of a nacreous layer, a prismatic layer, and periostracum (Fig. 3). Evidence that the layer shown between the periostracum and the nacre in Fig. 3 is actually prismatic was obtained by use of a polarizing microscope. By this means the crystals of the prismatic layer were seen to be oriented perpendicular to the laminated layer of the nacre. The identification of the prismatic layer proves that the shells of *M. transversum* agree with certain other pelecypod shells such as the Naiades (pearly fresh-water mussels) in possessing three fundamental layers in the shell.

The periostracum in a given section varies in thickness, measuring 0.003 to 0.009 mm in those observed in this study. In a given section, the prismatic layer varied in thickness also, measuring as much as 0.15 mm in the hinge region and as little as 0.027 mm in the middle of the valve where it seems to be the thinnest. The nacreous layer ranged from 0.009 to 0.069 mm in thickness, being the thinnest in the hinge and near the ventral edge. Of course, it must be taken into consideration that the measurements of each shell layer vary in thickness according to the size of the individual studied.

It is usually accepted that glands located on the ventral edge of the mantle secrete the prismatic layer and that the nacreous layer is secreted by cells of the general mantle surface. This may not be the case with the sphaerids since observations of the pyramidal cells and punctae tend to indicate a relationship with the prismatic layer. Pyramidal cells are not thought

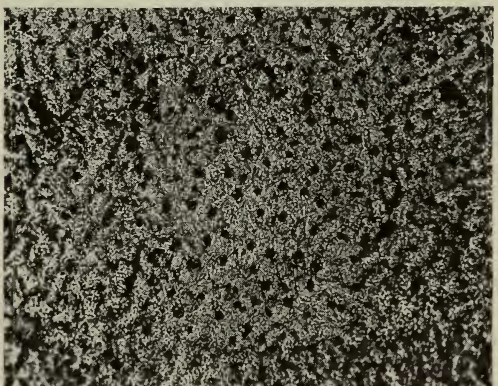

Fig. 2.—View of the inner surface of the shell showing the openings of the punctae into the shell. The openings appear as dark circles.

to be concerned with the formation of nacre since the pyramidal cells appear (grossly, that is) only after the nacreous layer is formed.

As mentioned before, the whole surface of the embryonic and young clam shell assumes a wrinkled appearance after the projections of the pyramidal cells protrude through the shell. This wrinkled condition was interpreted as being caused by the formation of raised, polygonal areas, each of which had a more or less centrally located projection. As stated before, these areas are thought to be the bases of the calcareous crystals of the prismatic layer. In other words, there appears to be one of the punctae with its corresponding pyramidal cell process piercing each polygonal prism of the prismatic shell layer. If this assumption is correct, then the pyramidal cells may be concerned with the secretion of the crystals of the prismatic layer. Such a method of secretory activity, however, does not conform to the generally accepted description of the secretion of the prismatic layer in other bivalves.

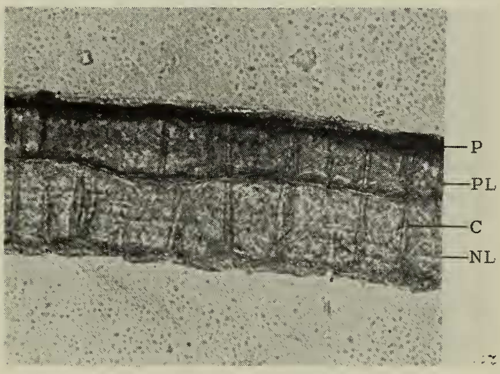

Fig. 3.—Ground section of the shell of a mature clam showing the periostracum (P), the prismatic layer (PL), the nacreous layer (NL), and the punctae (C) which traverse the calcareous layers of the shell. Some of the punctae appear discontinuous due to their curved shape. Note the laminations in the nacreous layer and the blurred line that separates the prismatic layer from the nacreous layer.

SUMMARY

Gross examination of the young and extramarsupial embryos of *Musculium transversum* shows that the pyramidal cells of the mantle send projections of material through the thin embryonic shell. The smallest individual exhibiting the projections measured 1.29 by 1.02 mm; the largest individual observed without the projections measured 1.63 by 1.25 mm.

The calcareous material of the shell surrounds the projections, forming the canals or punctae.

The punctae terminate in the periostracum either as plug-shaped structures or as pointed projections.

A study of the ground sections of the shells shows the presence of a nacreous layer, a prismatic layer, and a periostracum. The presence of a prismatic layer has been heretofore overlooked by students of this family.

The punctae and pyramidal cells of the relatively thin, fragile shell of the Sphaeriidae might possibly indicate a relationship with the secretion of the prismatic layer. Such a method of secretory activity, however, does not conform to the generally accepted description of the secretion of the prismatic layer in other clams.

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